REMARKS

The title of the invention has been amended to be more descriptive. The specification has been amended to insert a heading and to insert text from the figures.

Claim 24 has been amended to clarify the language of the claim, to delete part (e) and to specify that the DNA molecule which hybridizes to the DNA molecules of part (a) or part (b) under specified stringent hybridization conditions encodes a protein that contains seven conserved cysteine residues. Claim 24 has also been amended to set forth properties of the protein which finds support at the bottom of page 3 of the specification. Claim 24 has further been amended to correct a numbering of the nucleotides of one of the human MP121 fragments to correspond to that found in the claims of the issued parent patent and to correspond to the numbering for the corresponding mouse MP121 fragment. Claim 25 has been amended to specify the parts of the mature proteins of SEQ ID NOs:2 and 4 for which support can be found at pages 4 and 5 of the specification. Claim 26 has been amended to indicate that the other protein is a member of the TGF-β family for which support can be found at pages 10 and 11 of the specification.

New claim 29 has been added which finds support in original claim 25. New claim 30 has been added to specify the members of the TGF-β family and finds support at page 11, top paragraph. New claim 32 has been added to claim a monomeric protein comprising the protein of claim 24. Support for this claim can be found at page 31, first and second full paragraphs. New claims 33 and 34 have been added to claim pharmaceutical compositions of the proteins of new claims 31 and 32.

It is submitted that these amendments do not constitute new matter and their entry is requested.

The Examiner objected to the title, specification and figures. Applicants submit herewith a *Request for Approval of Drawing Changes* to delete figure descriptions from Figures 3-9. It is submitted that the amendments to the title and to the specification and the changes to the figures obviate these objections, and their removal is requested.

The Examiner rejected claims 24-28 under 35 U.S.C. §112, first paragraph, for lack of written description or enablement on several grounds. Each of these grounds of rejection under this section of the statute will be separately addressed.

In (4a), the Examiner contends that claims 24-28 lack written description. The Examiner contends that claim 25 encompasses the mature, secreted form of the protein of SEQ ID NO:2 and of the protein of SEQ ID NO:4, but the specification does not disclose the specific sequence of the mature protein recited in claim 25. Claim 25 has been amended to specify the parts of SEQ ID NOs:2 and 4 which correspond to the mature proteins. It is submitted that this amendment obviates this rejection, and its withdrawal is requested.

Although claim 25 has been amended to obviate the rejection, new claim 29 has been added. Claim 29 is of similar language as original claim 25 except that it contains the additional limitation that the mature proteins include the region of the seven conserved cysteine residues (see further discussion below concerning this region). It is submitted that the subject matter of claim 29 is adequately described in the specification as required by 35 U.S.C. §112, first paragraph.

First, the description of SEQ ID NO:2 and SEQ ID NO:4 on pages 4 and 5 shows what is understood by the term "mature protein." It is explained that the human protein begins between amino acid 217 and 240, preferably at 236 or 237, especially preferred at 237. The mouse protein also starts between amino acid 217 and 240, preferably at 237 or 236. Although the specification clearly describes the mature proteins with respect to MP121, the Examiner refers in the rejection to several proteins that are processed otherwise. According to the Examiner, it is not clear how the mature proteins are effected in the case of MP121. MP121 belongs to a protein family, i.e. the TGF-β superfamily and in a narrower sense to the activin/inhibin subfamily, whose members are all processed according to the same known scheme, so that what is meant by the statements of the present application would be completely evident to a skilled artisan. One skilled in the art would certainly not consider processing possibilities of the different proteins mentioned by the Examiner. For a general understanding, reference is made to page 2 of the specification, wherein it is stated:

> The members of this group have significant structural similarities. The precursor of the protein is composed of an amino-terminal signal sequence, a propeptide sequence and a carboxy-terminal sequence of 110-140 amino acids which is cleaved from the precursor and represents the mature protein.

Furthermore, it is known that the cleavage site of the mature protein within the TGF-β family members is found behind basic amino acids, which can be dibasic, tribasic or often is also a RXXR

sequence. See, e.g., Celeste et al. (*Proc Natl Acad Sci USA* 87:9843-9847, 1990) (copy attached) which states on page 9845:

> However, the presence of di- or tribasic amino acid sequence is not an absolute requirement for proteolytic processing, as a number of prohormones are known to be processed after single arginines that conform to a consensus cleavage sequence RXXR.

On page 5 of the specification, it is stated, "[M]embers of the TGF-β family are frequently cleaved behind a RXXR cleavage site in order to separate the mature part from the precursor" citing Özkaynak et al. (*J Biol Chem* 267:25220-25227, 1992) (copy to be provided as soon as obtained). Arginine occurs at positions 235 and 236 in human MP121creating a dibasic sequence. Thus, the preferred N-terminal amino acid is glycine at 237 similar with other TGF-β proteins. The mouse MP121 has the polybasic sequence RVRRR at position 232-236. Thus, the N-terminal amino acid could be arginine at 236 or glycine at 237, similar with the activins which are members of the TGF-β family most closely related to MP121. Figure 4 of Özkaynak et al. shows that both mature activins A and B also begin with glycine with a cleavage site of RRRRR and/or RIRKR preceding it. With respect to the disclosed broader region for the N-terminus of the mature protein, it is known that the N-terminus of TGF-β family members can be shortened without the activity being influenced. The Examiner's attention is directed to Sampath et al. (*J Biol Chem* 267:20352-62, 1992) (copy to be provided as soon as obtained) which discloses that shortened mature forms of OP-1 (also known as BMP7) are also active, as shown in Figure 2B and in the discussion at the end of page 20354 to page 20355, which states:

> Apparent degradation products migrating at approximately 15 kD in the same CHO hOP-1 preparations displayed four amino-terminal sequences corresponding to subunits with 119, 117, 116 and 114 amino acids of mature OP-1, whereas the 16 kD degradation products of BSC hOP-1 preparations presented two amino-terminal sequences corresponding to subunits with 116 and 114 amino acids (Figure 2B).

Furthermore, at page 20357 Sampath et al. states, "... indicated that the bone-forming potential of these NH2-terminally truncated forms of OP-1 were equivalent to that of intact mature OP-1 ..." This passage shows that in addition to the actual mature OP-1 with 139 amino acids other mature forms with 119, 117, 116 and 114 amino acids exist and are active. It is also known that it is

possible that 15 amino acids are missing at the N –terminus of inhibin and nevertheless the protein is still active. See, Vale et al. (*Handbook of Experimental Pharmacology* 95:211-248, 1990) cited on page 2 of the specification. Vale et al. at page 216 states, " ... deletion of the N-terminal 15 residues of the α-subunit in one purified fraction of an ovine inhibin dimer (...) does not destroy biological activity."

Similarly, it is expected that amino acids can be missing at the N-terminus of MP121. It is important that, as is known for other TGF-β family members, the conserved seven cysteine region remains which is essential for the correct fold of the proteins. Six of these cysteine residues form intramolecular cystine bridges and one cysteine forms an intermolecular cystine bridge to the next monomer to form the dimer. This seven cysteine region is often described as the TGF-β domain. See, Schlunegger and Grütter (Nature 358:430-44, 1992) (copy attached) which states at page 434, "[W]e therefore propose the general fold, including the TGF-β knot of all the proteins of the TGF-β superfamily to be one and the same ..." This folding is also disclosed in other articles well known to skilled artisans. Since these articles are all general knowledge of skilled artisans, a detailed explanation thereof has not been added to the specification. However, Figure 1 of the present application shows a sequence beginning with the first cysteine of the seven cysteine region of MP121 and compares it with similar members of the TGF-β family, i.e., activins/inhibins. In addition, page 1 of the present specification states, "[T]he mature protein contains the sequences that are conserved most, in particular seven cysteine residues, which are conserved among the family members." The mature protein is further defined on pages 6-7 of the specification as follows:

> Within the scope of the present invention, the term "mature protein" also encompasses functional partial regions of the complete protein which exhibit essentially the same biological activity and preferably those partial regions which include at least the region of the seven cysteines that are conserved in the TGF-β family. In this case, it is in particular possible that the N-terminus of the mature peptide is slightly modified ...

In view of these remarks, it is submitted that the subject matter of claim 29 is adequately described in the specification as required by 35 U.S.C. §112, first paragraph.

In (4b), the Examiner contends that claims 24-28 lack written description. The Examiner contends that claim 24 is a genus claim which encompasses protein variants of SEQ ID NOs:2 and

4. The Examiner contends that claim 25 encompasses naturally and non-naturally occurring variants of these proteins. The Examiner contends that the claims do not indicate what distinguishing attributes are shared by members of the genus. The DNA molecule of section (d) of amended claim 24 comprises a nucleotide sequence which hybridizes under specified stringent hybridization conditions to the DNA molecule of parts (a) or (b). Section (d) of claim 24 has further been amended to specify that the DNA molecule encodes a protein comprising an amino acid sequence containing seven conserved cysteine residues. The protein of claim 24 has mitogenic and/or differentiation-inductive activity. The proteins of the claims all share the following attributes: (1) they have mitogenic and/or differentiation-inductive activity; (2) they are encoded by the nucleic acids recited in parts (a) - (c) or by nucleic acids which hybridize under stringent hybridization conditions with the nucleic acids recited in parts (a) and (b); and (3) they all contain seven conserved cysteine residues. The hybridization is an important attribute which cannot be overlooked, as is the attribute of seven conserved cysteine residues. The claims do not encompass any proteins which do not contain seven conserved cysteine residues and which are encoded by nucleic acids which do not hybridize to the nucleic acids of parts (a) or (b) under stringent conditions. Applicants note that the language of amended claim 24 has been drafted in a manner to be consistent with the language of claim 1 of the parent application Serial No. 09/218,176, which the present Examiner allowed and which has matured into U.S. Patent No. 6,171,584.

In view of the amendment of claims 24 and 25 and the above remarks, it is submitted that the subject matter of claims 24-28 is adequately described in the specification as required by 35 U.S.C. §112, first paragraph. Withdrawal of this rejection is requested.

In (4c), the Examiner contends that claims 24-28 lack written description. The Examiner contends that section (d) of claim 24 encompasses homolog species of the proteins of SEQ ID NOs:2 and 4 as a result of the language "which differs from (a), (b) or (c) due to its origin from other mammals." This language has been deleted from amended claim 24. The DNA molecule of section (d) of amended claim 24 comprises a nucleotide sequence which hybridizes under specified stringent hybridization conditions to the DNA molecule of parts (a) or (b). Section (d) of claim 24 has further been amended to specify that the DNA molecule encodes a protein comprising an amino acid

sequence containing seven conserved cysteine residues. The protein of claim 24 has mitogenic and/or differentiation-inductive activity. The proteins of the claims all share the following attributes: (1) they have mitogenic and/or differentiation-inductive activity; (2) they are encoded by the nucleic acids recited in parts (a) - (c) or by nucleic acids which hybridize under stringent hybridization conditions with the nucleic acids recited in parts (a) and (b); and (3) they all contain seven conserved cysteine residues. Applicants note that the language of amended claim 24 has been drafted in a manner to be consistent with the language of claim 1 of the parent application Serial No. 09/218,176, which the present Examiner allowed and which has matured into U.S. Patent No. 6,171,584.

In view of the amendment of claim 24 and the above remarks, it is submitted that the subject matter of claims 24-28 is adequately described in the specification as required by 35 U.S.C. §112, first paragraph. Withdrawal of this rejection is requested.

In (4d), the Examiner contends that claim 24 [sic] lacks enablement. The Examiner contends that although the specification is enabling for the proteins of SEQ ID NOs:2 and 4, the specification is not enabling for the proteins of parts (d) and (e). Subpart (e) has been deleted in view of the amendment of part (d). All of the proteins of claim 24 have mitogenic and/or differentiation-inductive activity, as shown for the mature mouse and human MP121 proteins. Amended part (d) of claim 24 requires that the encoded protein contains the seven conserved cysteine residues. This disclosure, coupled with the disclosure of differing length mature peptides and multiple species of peptides, provide a representative number of examples of proteins falling within the scope of the claims. The specification further provides guidance for isolating additional peptides by using primers based on the human or mouse MP121 nucleic acid sequences. For example, the specification demonstrates that the mouse MP121 nucleic acid was obtained by using primers based on the human sequence. See Example 1, part 1.10. The proteins are readily determined from the obtained nucleic acid, which shows whether the encoded protein contains the seven conserved cysteine residues. The hybridization of the obtained nucleic acids to the nucleic acids of parts (a) or (b) under the specified stringent conditions is readily determined by one of ordinary skill in the art. The specification provides assays which can be used to determine the biological activity of the proteins which are obtained. Thus, it is submitted that no inventive contribution and no undue

experimentation is required to identify and use additional proteins encompassed by the claims. Furthermore, Applicants note that the language of amended claim 24 has been drafted in a manner to be consistent with the language of claim 1 of the parent application Serial No. 09/218,176, which the present Examiner allowed and which has matured into U.S. Patent No. 6,171,584.

In view of the amendment of claim 24 and the above remarks, it is submitted that the subject matter of claims 24-28 is fully enabled by the specification as required by 35 U.S.C. §112, first paragraph. Withdrawal of this rejection is requested.

The Examiner rejected claim 26 under 35 U.S.C. §112, second paragraph for being indefinite. It is submitted that the amendment of claim 26 obviates this rejection, and its withdrawal is requested.

The Examiner rejected claim 24 under 35 U.S.C. §102(b) as being anticipated by Forage et al. (1986). In view of the amendments to claim 24 and since the cited subsequence of Forage et al. does not contain seven conserved cysteine residues, it is submitted that claim 24 is not anticipated by Forage et al. Withdrawal of this rejection is requested.

The Examiner rejected claims 24 and 27 under 35 U.S.C. §102(b) as being anticipated by Mason et al. (US 4,798,885). In view of the amendments to claim 24 and since the cited sequence of Mason et al. which contains seven conserved cysteine residues does not contain sufficient similarity to hybridize under stringent conditions with the nucleotide sequences set forth in the claims, it is submitted that claims 24 and 27 are not anticipated by Mason et al. Withdrawal of this rejection is requested.

In view of the above amendments and remarks, it is believed that the present claims satisfy the provisions of the patent statutes and are patentable over the cited prior art. Reconsideration of

the application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned to expedite the prosecution of the application.

RESPECTFULLY SUBMITTED,					
Name and Reg. No.	Jeffrey L. Ihnen, Registration No. 28,957				
Signature				*Date*	21 February 2003
Address	Rothwell, Figg, Ernst & Manbeck 1425 K Street, N.W., Suite 800				
City	Washington	*State*	D.C.	*Zip Code*	20005
Country	U.S.A.	*Telephone*	202-783-6040	*Fax*	202-783-6031

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Attachment: Marked-up Copy of Amendments
Copy of Celeste, A.J. et al., *Proc Natl Acad Sci USA* 87:9843-9847 (1990)
Copy of Schlunegger, M.P & Grütter, M.G., *Nature* 358:430-434 (1992)

Marked-up Copy of Amended Title

~~New growth/differentiation factor of the TGF-β Family~~ MP121, A GROWTH/ DIFFERENTIATION FACTOR OF THE TGF-β FAMILY

Marked-up Copy of Amended Specification - Second Full Paragraph, Page 6

Figure 3 shows a diagram of a Western blot using chicken antibodies against human MP121. Lane 1 shows *E. coli* cells transformed with pBP4MP121His under reducing conditions (1% β-mercaptoethanol. Lane 2 shows cell culture supernatant of NIH-3T3 cells after infection with recombinant viruses (with inserted MP121 cDNA) under reducing conditions (1% β-mercaptoethanol). Lane 3 shows cell culture supernatant of NIH-3T3 cells after infection with recombinant viruses (with inserted MP121 cDNA) under non-reducing conditions. Lane M shows prestained protein molecular weight markers having the stated apparent molecular weights (Gibco BRL #26041-020).

Marked-up Copy of Amended Specification - Third Full Paragraph, Page 6

Figure 4 shows the expression of MP121 compared to activin β_A and β_B in various mouse tissues and is an autoradiogram after gel analysis of an RNASE protection assay using specific probes against activin βA (βA), activin βB (βB), MP121 and against GAPDH for the control. Total RNA was tested which has been isolated from various mouse tissues (Lane 1: brain; Lane 2: heart, Lane 3: kidney, Lane 4: liver, Lane 5: lung, Lane 6: muscle, Lane 9: ovary, Lane 10: spleen, Lane 11: testes) from embryonic stem cells (Lane 12: CJ7) and from yeast (Lane 13) as a control. No RNA was used in Lane 14 as a control. The unprotected antisense RNA probes used for the hybridization are applied in lanes 8 and 15 and the expected fragment size is indicated in brackets in the right margin. The bands of the protected fragments are labeled in the left margin. PBR322

restricted with Map 1 (Biolabs #303) and end-labeled with γ-32p-ATP (Amersham) was used as the marker (Lane 7).

Marked-up Copy of Amended Specification - Fourth Full Paragraph, Page 6

Figure 5 shows a positive influence on the survival of dopaminergic neurons by treatment with partially purified MP121. The number of TH-immunoreactive dopaminergic neurones surviving after isolation from the mesencephalon of rat embryos (E14) after 8 days culture is shown. The effect of 20 ng/ml partially purified MP121 was tested compared to the equivalent amount of partially purified control supernatant (wt) as well as untreated neurones (control: medium containing 0.3% acetonitrile). The mean ± SEM from a triple determination is shown.

Marked-up Copy of Amended Specification - Fifth Full Paragraph, Page 6

Figure 6 shows a Western blot using rabbit antibodies against human MP121. Lane 1 shows cell culture supernatant of HepG2 cells after infection with recombinant viruses (with inserted MP121 cDNA) under non-reducing conditions. Lane 2 shows cell culture supernatant of HepG2 cells after infection with wildtype viruses under non-reducing conditions. Lane 3 shows prestained protein molecular weight markers having apparent molecular weights of 15.5, 18.2, 27.8, 43.8 and 71.5 kD (Gibco BRL #26041-020), indicated schematically. Lane 4 shows cell culture supernatant of HepG2 cells after infection with recombinant viruses (with inserted MP121 cDNA) under reducing conditions. Lane 5 shows cell culture supernatant of HepG2 cells after infection with wildtype viruses under reducing conditions.

Marked-up Copy of Amended Specification - Sixth Full Paragraph, Page 6

Figure 7 shows the stimulation of nerve fibre outgrowth from the embryonic retina by treatment with partially purified PM121. Dark field microscopy of living cultures shows nerve fibre

outgrowth from explanted chicken retina after 4 days in culture in the presence of 5 ng/ml partially purified MP121.

Marked-up Copy of Amended Specification - Seventh Full Paragraph, Page 6

Figure 8 shows that various concentrations of partially purified MP121 can inhibit EGF induced DNA synthesis in hepatocytes.

Marked-up Copy of Amended Specification - Eighth Full Paragraph, Page 6

Figure 9 shows the influence of various concentrations of partially purified MP121 on erythroid differentiation measured by the percentage of dianisidine positive cells.

Marked-Up Copy of Amended Claims

24 (amended). An isolated protein of the TGF-β family which has mitogenic and/or differentiation-inductive activity and is coded by a DNA molecule selected from the group consisting of

(a) a DNA molecule comprising the nucleotide sequence shown in the SEQ ID No:1, or the following fragments: nucleotides 128-1183, nucleotides 836-1183, nucleotides 128-835, and nucleotides ~~886~~ 866-1183;

(b) a DNA molecule comprising the nucleotide sequence shown in the SEQ ID No:3, or the following fragments: nucleotides 131-1186, nucleotides 839-1186, nucleotides 131-838, and nucleotides 869-1186;

(c) a DNA molecule encoding the amino acid sequence encoded by (a) or (b); and

(d) a DNA molecule comprising a nucleotide sequence which ~~differs from (a), (b) or (c) due to its origin from other mammals wherein said nucleotide sequence~~ hybridizes with one of the DNA molecules ~~sequences~~ from (a)~~,~~ and (b)~~, or (c)~~ under stringent hybridization conditions in 6x SSC at 62-66° C followed by one hour wash with 0.6x SSC and 0.1% SDS at 62-66°~~; and~~

~~(e) a nucleotide sequence which hybridizes with one of the sequences from (a), (b), (c) or (d) under stringent hybridization conditions in 6x SSC at 62-66° C followed by one hour wash with 0.6x SSC and 0.1% SDS at 62-66°~~ C and which encodes a protein comprising an amino acid sequence containing seven conserved cysteine residues.

25 (amended). The protein according to claim 24, wherein said protein has an amino acid sequence selected from the group consisting of SEQ ID NO:2; SEQ ID NO:4; a ~~the~~ part of SEQ ID NO:2 corresponding to the mature protein which starts with one of amino acids 217-240; a ~~the~~ part of SEQ ID NO:4 corresponding to the mature protein which starts with one of amino acids 217-240; and sequences containing conservative substitutions of the amino acids shown in SEQ ID NO:2~~, and~~ SEQ ID NO:4 or the said parts thereof.

26 (amended). A heterodimeric protein comprising a monomer of the protein of claim 24 and a monomer of another protein from the ~~superfamily with a "cysteine knot motif"~~ TGF-β family.